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(54) Title: ANTICANCER VACCINE COMPRISING IL6/IL6 RECEPTOR TRANSFECTED CELLS

(57) Abstract

Genetic anticancer vaccine for stimulation of patient's immune system to eradicate cancer, particularly malignant melanoma. The objective of the invention is genetic modification of allogeneic cancer cells by insertion of the two genes, one encoding human interleukin 6 and the other encoding soluble interleukin 6 receptor, which will be administered to patients.

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ANTICANCER VACCINE COMPRISING IL6/IL6 RECEPTOR TRANSFECTED CELLS

The objective of the invention is genetic anticancer vaccine for gene therapy of human neoplastic diseases particularly malignant melanoma.

Concept of so called genetic cellular vaccines is based on genetic modification of autologous (patient's own) or antigenetically related (allogeneic) cancer cells in order to activate patient's immunologic system to eliminate cancer. Autologous (obtained from each patient to be treated) and/or allogeneic (established cancer cell lines) genetically modified cancer cells are irradiated and injected subcutaneously to the patient. Until now cancer (autologous or allogeneic) cells in order to provide costimulatory signal for patient's own immune system have been genetically modified by insertion of various genes encoding: interleukin (IL) 2 [allogeneic cells; (1)], IL-4 (2), IL-7 (3), tumor necrosis factor [TNF; (4)], interferon gamma (5) or macrophage-granulocyte colony stimulating factor [GM-CSF) (6)] (autologous cells).

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5. Siegler H.F, A Phase I trial of Human Gamma Interferon-Transduced Autologous Tumor Cells in Patients with Disseminated Malignant Melanoma. **Human Gene Therapy**, 5:761-773, 1994.

6. **Patent No WO 9418995** (September 1, 1994): Retrovirus transduced tumor cell for producing immunomolecule - interleukin 2, interferon gamma, colony stimulating factor, adhesion molecule, tumor-associated antigen, etc, antisense RNA expression by packaging cell culture for eg tumor gene therapy.

The objective of the invention is genetic modification of human malignant melanoma cell line which is HLA-A1 and HLA-A2 positive by introduction into the cells two genes (cDNA) coding human IL-6 and soluble IL-6 receptor (sIL-6R).

SIL-6R was constructed by replacement of cytoplasmic and transmembrane domains of the membrane receptor by translational stop codon using polymerase chain reaction (PCR) with primer 5'CGGATCCGTCGACTAATCTTGGCACTGGGAGGCTTG3'. Moreover, signal peptide was replaced by translational start codon ATG using PCR with primer 5'GGGGACATGTTAGCCCCAAGGCGCTGCCCT3', introducing methionine as a first aminoacid of sIL-6R.

Another objective of the invention is a vaccine containing autologous cancer cells and genetically modified allogeneic cancer cells. Combination of autologous and allogeneic cells will increase immunogenicity and effectiveness of the vaccine. In this variant of the vaccine autologous cells do not require genetic modification. Products of introduced genes will be supplied by allogeneic cells and their biological effect will be provided by "by stander effect".

EXAMPLES OF APPLICATION OF THE INVENTION

1. From the melanoma patient (HLA-A1 and/or HLA-A2 positive) a cancer metastatic focus will be surgically excised. Obtained tissue will be minced, cells enzymatically isolated and either frozen in liquid nitrogen or cultured in vitro in typical conditions. After obtaining in culture required number of cells they will be mixed (1 : 1) with genetically modified allogeneic cells. If propagation of autologous cells in vitro will not be possible cells frozen in liquid nitrogen will be thawed and used. Then the mixture (5×10^7 cells per injection) will be irradiated using a total dose of 100 Gy and subcutanously administered to the patient. Four injections will be administered in two weeks intervals followed by three injections once a month. If necessary injections will be continued in two months intervals.

2. In some melanoma patients excision of metastases will not be possible due to the advancement of the disease or localization of lesions. In such cases allogeneic vaccine will be applied. Genetically modified cells (5×10^7) will be irradiated and administered as described above.

CLAIMS

1. Genetic anticancer vaccine, containing genetically modified allogeneic cancer cells, characterized in, that allogeneic cells contain two genes, one encoding interleukin 6 (IL-6), and the other encoding interleukin 6 soluble receptor (sIL-6R).

2. Genetic anticancer vaccine according to claim 1. characterized in, that contains gen (cDNA) for human IL-6 and gen (cDNA) for human sIL-6R, while sIL-6R is a modified membrane receptor in which cytoplasmic and transmembrane domains were replaced by translational stop codon, and signaling peptide was replaced by translational start codon.

3. Genetic anticancer vaccine, containing autologous cancer cells, characterized in, that it also contains genetically modified allogeneic cancer cells, while content of allogeneic cells can not be lower then 50% and can not exceed 70%.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/00

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
O,X	ANNALS OF THE NEW YORK ACADEMY OF SCIENCES,VOLUME 762.INTERLEUKIN-6-TYPE CYTOKINES., 19 - 22 June 1994, POZNAN,POLAND, pages 361-387, XP000602025 MACKIEWICZ ET AL: "INTERLEUKIN-6-TYPE CYTOKINES AND THEIR RECEPTORS FOR GENE THERAPY OF MELANOMA" see the whole document & ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, vol. 0, no. 0, 1995, --- -/--	1-3

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CYTOKINE, vol. 7, no. 2, February 1995, pages 142-149, XP000602017 MACKIEWICZ ET AL: "SOLUBLE INTERLEUKIN 6 RECEPTOR IS BIOLOGICALLY ACTIVE IN VIVO" see the whole document ---	1-3
A	EP,A,0 538 952 (YEDA RES & DEV) 28 April 1993 see page 2, line 3 - line 6 ---	1-3
P,X	HUMAN GENE THERAPY, vol. 6, June 1995, pages 805-811, XP000602016 MACKIEWICZ ET AL: "GENE THERAPY OF HUMAN MELANOMA.IMMUNIZATION OF PATIENTS WITH AUTOLOGOUS TUMOR CELLS ADMIXED WITH ALLOGENEIC MELANOMA CELLS SECRETING INTERLEUKIN 6 AND SOLUBLE INTERLEUKIN 6 RECEPTOR" see page 806,'objectives' -----	1-3

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0538952	28-04-93	IL-A-	99821	23-07-96
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